Liposomes: Critical Formulation Parameters

Frank Martin, Ph.D.
ALZA Corporation

Liposome Pharmacology: Critical Influences

- Size
- Structural lipid
- Surface properties
- Drug
  - Pharmacology
  - Encapsulation stability
  - Release kinetics

Drug Itself

- Drug class (cytotoxic, biologic, anti-infective)
- Intrinsic PK, safety profile
  - Plasma clearance, tissue distribution
  - Dose-related toxicities (single-dose, cumulative)
  - Toxic sites
- Target
- Encapsulation
  - Payload
  - Stability
  - Release kinetics

Liposome Size

- Physical aspects
  - Size distribution – outliers
  - Size growth
    - in suspension
    - during/after reconstitution
  - Size measurement issues
- Safety issues
  - Micro-capillary occlusion
  - MPS saturation
- Extravasation window
- Sterilization by filtration

Structural Lipid Matrix

- Influence of cholesterol
  - Condenses bilayer
  - Stabilizing effect
  - Critical proportion
- Fatty acids
  - Phase behavior
    - Liquid crystal vs. fluid
    - Boundary effects
  - Tendency to oxidize
    - Chain reaction
- Headgroup

Surface Properties

- "Naked" lipid
  - Protein adsorption
    - Opsinization – rate of MPS uptake
    - Destabilization – drug leakage
    - Flocculation
- Surface charge
  - Sign
  - Density
- Surface coating
  - Carbohydrate
  - Polymer

Case Study: Doxil

Comparison of Design Features and Pharmacology of Liposomal Anti-tumor Formulations

Pharmacology of Cytotoxic Drugs

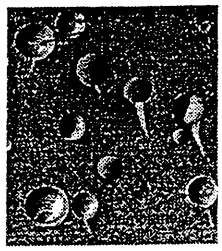
- Relative lack of control over tissue distribution, pharmacokinetics
- Drugs distribute based on chemical properties
  - solubility, charge
  - molecular weight
  - protein binding
- Only level of control is "input" rate (dose intensity, infusion schedule)
- Liposomes intended to favorably influence tissue distribution/kinetics

Pharmacology Depends on Design

- MPS Targeting
  - better tolerance – attenuates peak
  - but no opportunity for targeting – liposomes cleared too quickly to reach tumor
- MPS Avoiding
  - potential for reaching tumor – circulates longer
  - but must keep drug "on-board" while liposome moves through bloodstream
  - must release drug once in tumor

MPS-Targeted Liposomes

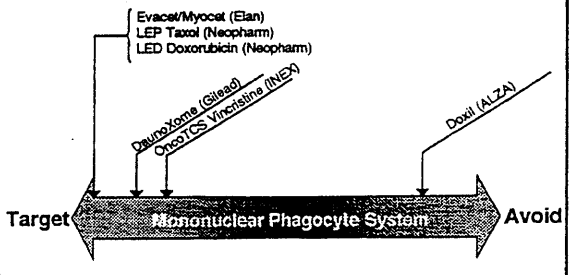
- Proven useful to improve safety of drugs with "peak" toxicities
  - doxorubicin (eg. Evacet/Myocet)
  - daunorubicin (eg. DaunoXome)
  - amphotericin B (eg. Ambisome)
- Cleared rapidly by macrophages (liver and spleen)
- Creates "depot" -- mimics a slow infusion
- Limited opportunity for targeting



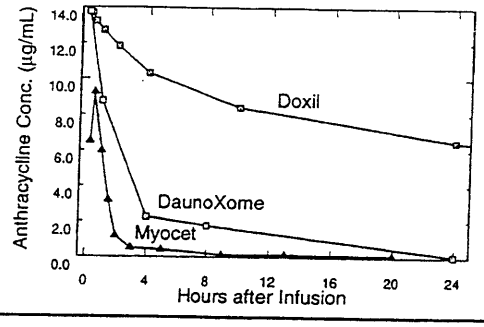
MPS-Avoiding Liposomes

- Must be stable in blood
  - polymer layer keeps proteins from binding
  - less drug leakage after injection
- Clearance by macrophages slow
- Able to "passively" target encapsulated drugs to sites of disease
- Useful to improve ***activity*** of drugs

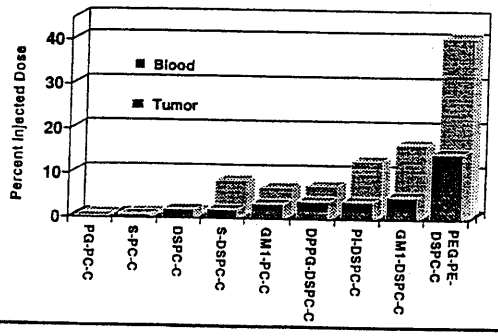
Relative Rates of MPS Clearance



Relative Rates of Clearance of Anthracycline Liposomes From Blood



Correlation Between Tumor Uptake and Liposome Circulation Time

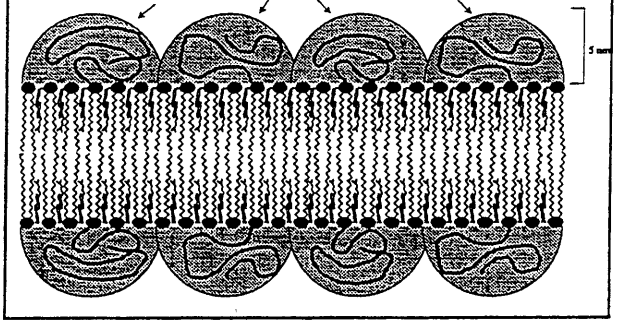


Doxil

- Size selected to balance payload, clearance, extravasation potential, ease of sterilization
  - 10-15K molecules of doxorubicin per liposome
- Pegylated for long circulation times (half life – 70 hours)
- Passive accumulation in tumors through "leaky" blood vessels
- Lipid matrix selected for plasma stability, and *in situ* release of drug

DOXIL Liposome Structure

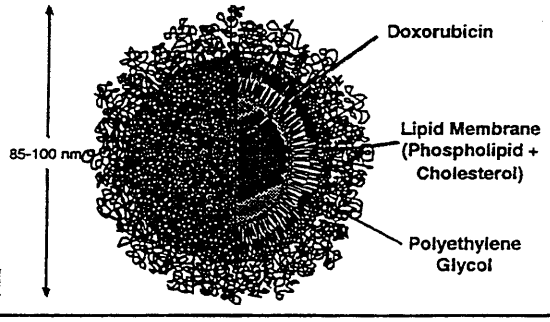
Overlapping "Mushroom" Model Best Describes Orientation of PEG Groups



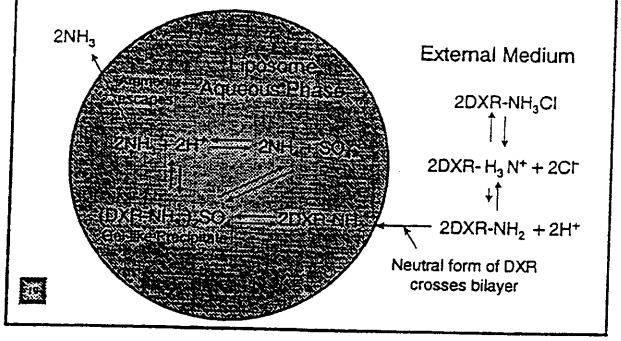
Kinetic Origin of the "Stealth" Effect

- PEG layer slows protein adsorption (opsonization)
  - less total protein bound
  - fewer protein species bound
- Recognition/uptake by MPS slowed
- Lipid transfer between outer monolayer of liposome and lipoproteins, formed elements and endothelial cells is slowed
- Slower clearance and maintenance of structural integrity provides time for liposomes (with encapsulated drug) to extravasate in tissues with compromised endothelial barriers (i.e., tumors)

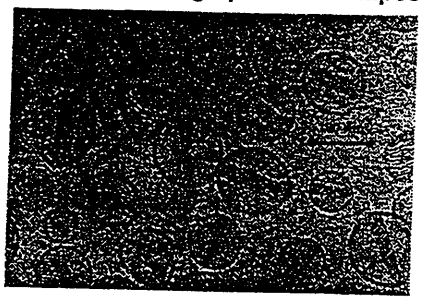
Structure of Doxil



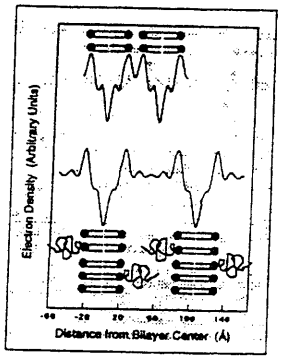
Active Loading of Doxorubicin by Ammonium Sulfate Gradient Method



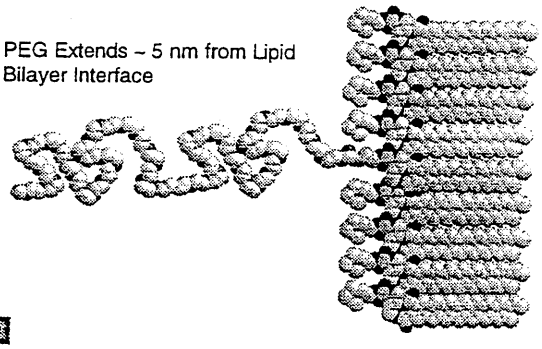
Cryo Electron Micrograph of Doxil Liposomes



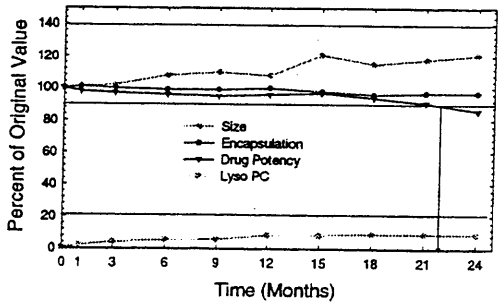
Electron density profiles for phosphatidyl choline bilayers in the absence (top) and presence (bottom) of 5m% DSPE-PEG_{2000}



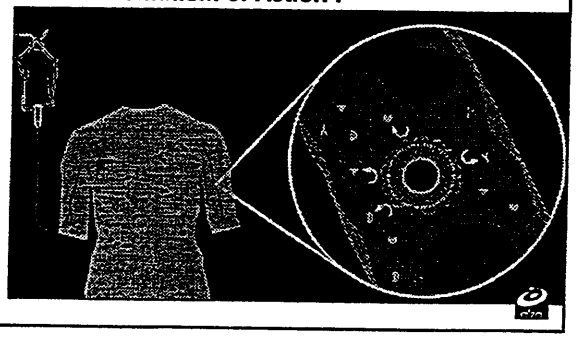
PEG Extends ~ 5 nm from Lipid Bilayer Interface



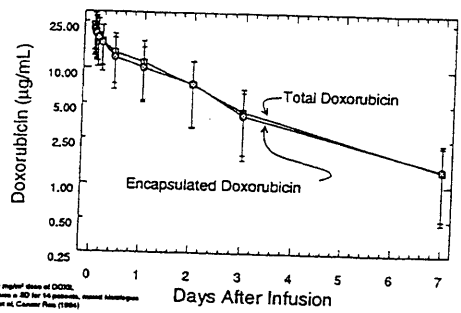
Doxil Stability (Aqueous Suspension, 8°C)



Doxil Mechanism of Action I

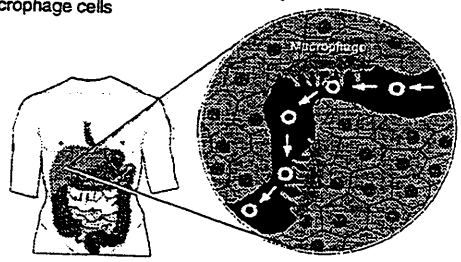


Drug Remains Encapsulated After Doxil



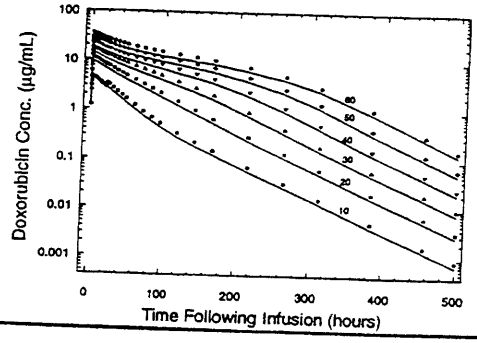
Doxil Mechanism of Action II

Prolonged circulation: Slower uptake by liver macrophage cells



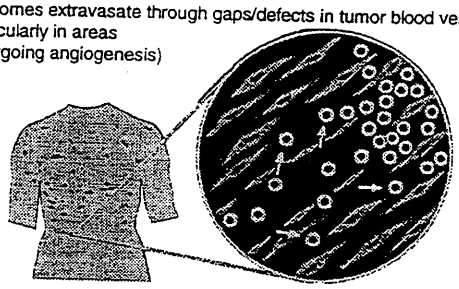
Doxil Pharmacokinetics

(Single dose over clinically relevant range 10-60 mg/m²)

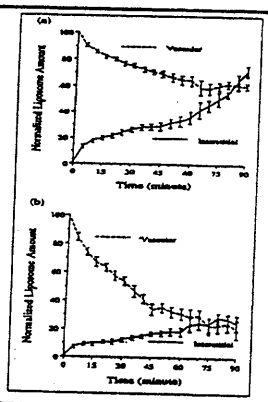


Doxil Mechanism of Action III

Liposomes extravasate through gaps/defects in tumor blood vessels (particularly in areas undergoing angiogenesis)

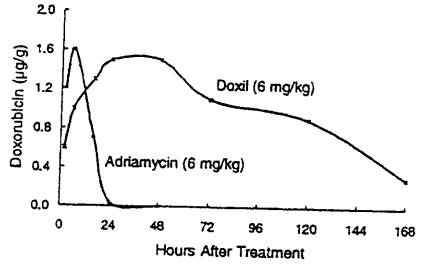


Fluorescence intensities vs. time in blood vs. interstitium of implanted tumor after administration of pegylated (top) and non-pegylated liposomes (bottom)

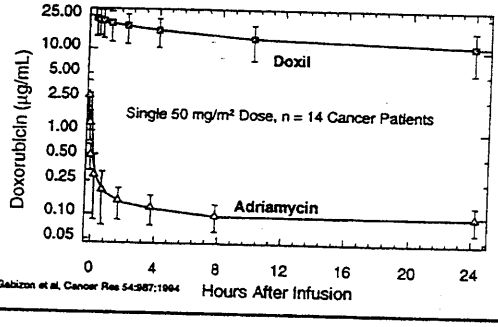


Pegylated Liposome Extravasation in Angiogenic Blood Vessels

"Area under the Curve" of Doxil Vs. Adriamycin in Human Pancreatic Carcinoma Xenografts

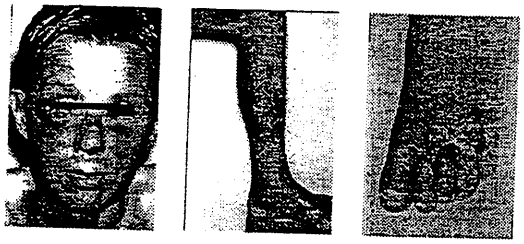


Plasma Levels of Doxorubicin: Doxil vs. Adriamycin

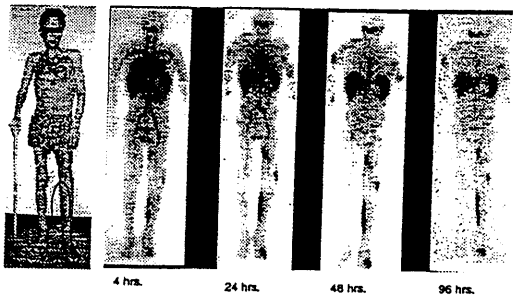


Gabizon et al, Cancer Res 54:987;1994

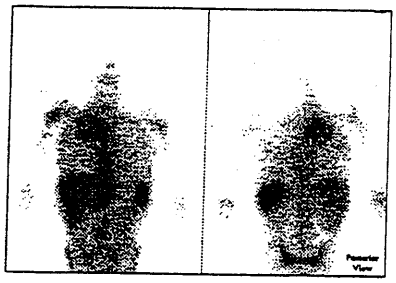
KS Patient - Prior to Injection



Serial Gamma Scintigrams of KS Patient after Pegylated Liposomes Containing ^{111}In-DTPA

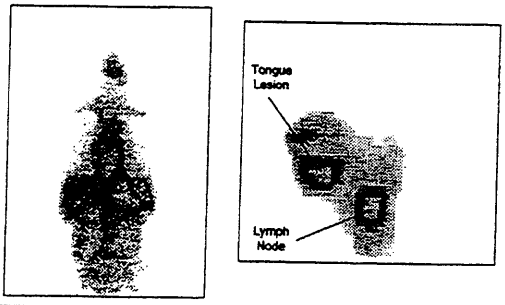


Gamma Scintigram of Lung Cancer Patient 96 Hrs. after Pegylated Liposomes Containing ^{111}In-DTPA



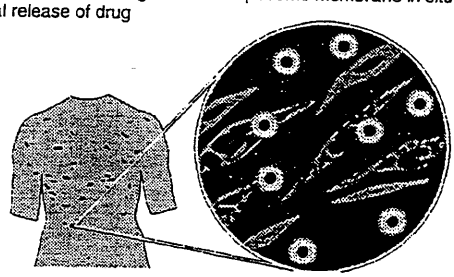
Gamma Scintigrams of Patient with T4 Squamous Cell Carcinoma of the Tongue

(96 Hrs. after Pegylated Liposomes Containing ^{111}In-DTPA)

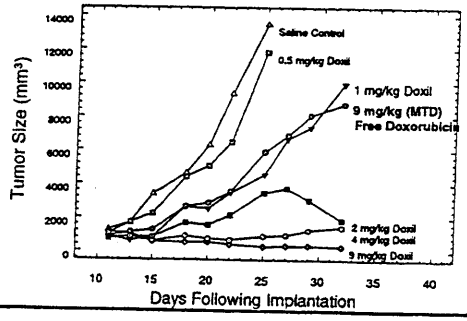


Doxil Mechanism of Action IV

Physical/chemical degradation of liposome membrane *in situ* and local release of drug



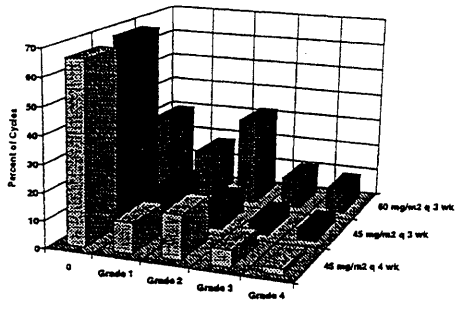
Therapeutically Equivalent Dose of Doxil Vs. Adriamycin in Lewis Lung Tumor Model



Doxil vs. Doxorubicin

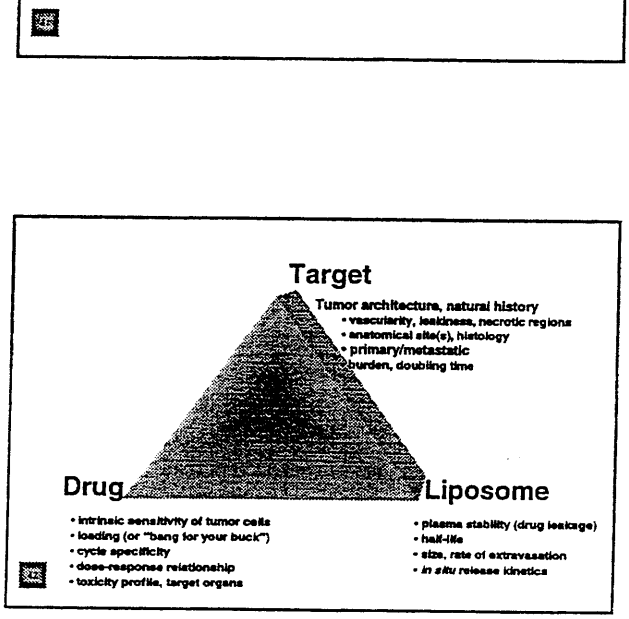
- Shift in safety profile
  - HF syndrome limits dose rate to 10-12.5 mg/m² per week
  - at this dose, other toxicities low
- Shift in efficacy
  - appears to be as active in doxorubicin-sensitive histologies (breast, lymphoma, multiple myeloma)
  - may be more active in KS, ovarian, head&neck
- Net clinical benefit
  - active at dose rate ½ that of doxorubicin
  - patients exposed to less drug less frequently
  - more active than doxorubicin in some histologies

Relationship Between Incidence and Severity of Neutropenia and Doxil Dosing Schedule



Doxil Experience

- Reveals limitations/ promise of passive targeting
- It's all true; clinical performance depends on delicate balance among:
  - target
  - drug
  - liposome



Critical Parameters for Successful Passive Targeting to Tumors

- High drug payload
- Stability *in vitro* (no drug leakage, aggregation)
- Drug retention in blood stream (during tissue distribution phase)
- Extravasation
  - long plasma residence time (days)
  - size (<400 nm)
- *In situ* release in tumor (tailor drug loading/barrier properties to drug)

Prospects

- Rational drug selection
  - mate deficiencies of drug with benefits of liposome
  - not a long list – but candidates do exist
    - antisense
    - camptothecin analogues
    - angiogenesis inhibitors
- Rational drug design
  - loadable and stable (more is better)
  - mechanism (tailored to *in situ* release rate)
  - tumor sensitivity (clinical need)
- Ligand targeting offers promise of more selectivity, but
  - target cell must be accessible, and
  - uptake must provide clinically demonstrable benefit over non ligand-bearing counterpart